PULMONARY BIOMARKERS BASED ON ALTERATIONS IN PROTEIN EXPRESSION FOLLOWING EXPOSURE TO ARSENIC

R. Clark Lantz[1,2], Brandon J. Lynch[1], Scott A. Boitano[2,3] and Jefferey L. Burgess[2,4]

Department of Cell Biology & Anatomy[1], Southwest Environmental Health Science Center[2], Arizona Respiratory Center[3] and Environmental and Community Health[4], University of Arizona, Tucson, AZ

ABSTRACT

Environmental exposure to arsenic (As) results in multiple adverse effects which can be characterized through evaluation of alterations in protein expression. We have used proteomics to evaluate and identify biomarkers of chronic environmental exposure to arsenic by evaluating large numbers of proteins simultaneously. Mice were administered 50 ppb of arsenic in their drinking water for 4 weeks. Proteins in the lung lining fluid were obtained through bronchoalveolar lavage (BAL). Proteins were precipitated prior to separation using 2-D gel electrophoresis. Gels from four control and four arsenic treated mice were examined for consistent alterations in proteins. Proteins with altered levels of expression between the control and treated groups were cut from the gels, digested with trypsin and subjected to mass spectrometry for peptide and putative protein identification. Proteins that were seen to be present in the BAL of control animals while absent in the treated animals include: glutathione-S-transferase omega-1 (GST omega-1), phosphatase and tensin homolog (PTEN), alpha-1-antitrypsin and receptor for advanced glycation end products (RAGE). In addition, peroxiredoxin-6 was up-regulated in arsenic exposed animals compared to controls. Previous investigators have identified GST omega-1 as an important arsenic metabolizing enzyme, while PTEN is an important tumor suppressor gene. Peroxiredoxin-6 is important in oxidative stress and surfactant metabolism. RAGE is a multiligand member of the immunoglobulin superfamily of cell surface receptors originally described as a receptor for products of glycation and oxidation of proteins and lipids. The RAGE-ligand axis is being recognized for its role in several chronic diseases, such as diabetes, atherosclerosis, coronary artery disease and lung cancer. We therefore chose to determine whether arsenic-induced alterations in RAGE seen in our animal models were also present in humans exposed to arsenic in their drinking. Lung induced-sputum samples were collected from 57 individuals living in Ajo, AZ (N=34) (tap water As ~20 ppb) and in Tucson, AZ (N=23) (tap water As ~5 ppb). A first morning void urine sample was collected and analyzed for As+3, As+5, MMA, and DMA as a measure of arsenic exposure. RAGE levels in sputum were determined using commercially available ELISA kits. Regression analysis demonstrated a significant negative correlation (p=0.016) between sputum levels of RAGE and total urinary inorganic arsenic. This is a similar response to that seen in our animal models. Therefore, use of animal models combined with proteomics approaches provide an unbiased determination of important biomarkers that may be related to human disease. Supported in part by USEPA R832095 and by NIH P30ES06694.

OBJECTIVES

To evaluate and identify biomarkers of chronic environmental exposure to arsenic by evaluating large numbers of proteins simultaneously.

METHODS

ANIMAL STUDIES

- Adult male mice (C57Bl/6) were administered 10 or 50 ppb of arsenic in their drinking water for 4 weeks.
- Soluble proteins in the lung lining fluid were obtained through bronchoalveolar lavage (BAL).
- Proteins were acetone precipitated and processed through BioRad cleanup kit prior to separation using 2-D gel electrophoresis.
- Proteins were separated on 12.5% acrylamide gels with mass range of 15 to 250 KD and PI range from 5-8. Equal amounts of protein from individual animals were run on separate gels. Gels from three control and three arsenic treated mice were examined for consistent alterations in proteins.
- Proteins with altered levels of expression between the control and treated groups were cut from the gels, digested with trypsin and subjected to mass spectrometry for peptide and putative protein identification.

HUMAN STUDIES

- For each participant in Ajo, AZ and Tucson, AZ :
- Tap water was collected.
- A first morning void urine sample was collected.
- An HPLC-ICP-MS speciation method was modified for the measurement of arsenic in water and urine.
- Sputum induction was performed.
- Sputum supernatant samples were analyzed in duplicate for levels of RAGE using ELISA (R&D Systems, Minneapolis, MN).

RESULTS

IDENTIFICATION OF ALTERED PROTEIN EXPRESSION IN LUNG LAVAGE FLUID USING 2-D GELS

Proteins were isolated from lung bronchoalveolar lavage of control and 50 ppb arsenic treated mice.

Differentially expressed spots were cut out of gels, trypsin digested and subjected to identification using mass spectrometry.

PROTEINS DOWN REGULATED BY ARSENIC

1. Receptor for aged glycation end products (RAGE), important in chronic inflammatory diseases such as diabetes, coronary artery disease and arthritis.
2. Glutathione-S-transferase omega-1 (GST omega-1), an arsenic metabolizing enzyme.
3. Phosphatase and tensin homolog (PTEN), a tumor suppressor gene.
4. Contraspin (control spot 10), α-1-antitrypsin isoform 3K
5. Apolipoprotein A-IV

PROTEINS UP REGULATED BY ARSENIC

1. Peroxiredoxin-6, a bifunctional protein with both GSH peroxidase and phospholipase A_2 activity.

ALTERED PROTEIN EXPRESSION IN HUMAN INDUCED-SPUTUM

- Data obtained from exposed animals were used to guide analysis of arsenic-induced changes in humans.
- RAGE was selected for evaluation in humans, because if its role in chronic diseases, such as diabetes, atherosclerosis, and lung cancer.
- Lung induced-sputum samples were collected from 57 individuals living in Ajo, AZ (N=34) and in Tucson, AZ (N=23).
- RAGE concentrations in induced sputum were determined using commercially available ELISA kits.
- A first morning void urine sample was collected and analyzed for As+3, As+5, MMA, and DMA as a measure of arsenic exposure.

TABLE 1. CONCENTRATION OF ARSENIC SPECIES IN WATER & URINE AND SPUTUM RAGE LEVELS BY TOWN

	Variable	Ajo Mean ± S.D	Tucson Mean ± S.D	Sig. *p*-value
Water (n)		29	21	
	Total Water As (μg/L)	20.2 ± 3.6	4.0 ± 2.4	<0.001
Urine (n)		34	22	
	Total Urinary As (μg/L)	27.4 ± 18.5	15.8 ± 15.7	0.019
	Total Urinary Inorganic As (μg/L)	28.2 ± 20.5	13.0 ± 13.7	0.004
	Urinary $As^{3+} + As^{5+}$ (μg/L)	8.6 ± 9.0	6.0 ± 10.1	0.328
	Total Urinary MMA (μg/L)	2.9 ± 3.9	1.1 ± 1.2	0.003
	Total Urinary DMA (μg/L)	16.7 ± 11.7	5.8 ± 4.8	<0.001
	Urinary MMA / ($As^{3+} + As^{5+}$)	0.73 ± 0.67	2.12 ± 3.79	0.572
	Creatinine Adjusted Urinary As (μg/g)†	32.3 ± 13.3	16.1 ± 10.3	<0.001
Sputum (n)		34	23	
	RAGE (pg/ml)	5473 ± 25824	1336 ± 1260	0.662
	RAGE (pg/ml) excluding regression outliers	1192 ± 962	1396 ± 1254	0.844

Table 2. Regression Model for sputum RAGE concentration

N	Adjusted R^2	Outcome Variable	Predictor Variables	β-Coefficient	p-Value
47	0.210	ln RAGE	Town	0.011	0.973
			Diabetes	0.761	0.079
			BMI	0.091	0.002
			Total Urinary Inorganic As	-0.021	0.016

- Sputum RAGE concentrations were not significantly different by town (Table 1).
- There was a marked overlap of urinary total inorganic arsenic concentrations across the towns.
- Therefore sputum RAGE concentrations were compared with urinary total inorganic arsenic concentration combining both towns (Figure 1).

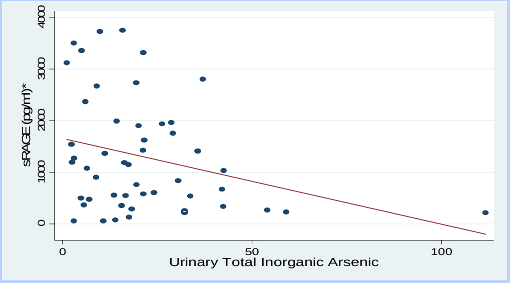

Figure 1. Plot of individual urinary total inorganic arsenic versus induced sputum levels of RAGE. There is a significant negative association between urinary total inorganic arsenic concentrations and RAGE (straight line in Figure 1). The straight line is derived from the regression model presented in Table 2.

CONCLUSIONS

- Use of animal models combined with proteomics approaches provide an unbiased determination of important biomarkers that may be related to human disease.
- Similar responses were seen in both animal models and in humans for RAGE expression.
- This suggests that alteration of this protein may play an important role in arsenic-induced lung injury.
- Proteomics techniques can be used to identify unique, previously unknown, biomarkers.

Supported by NIEHS Center Grant ES006694 and by and by USEPA R832095.